Proteoglycans

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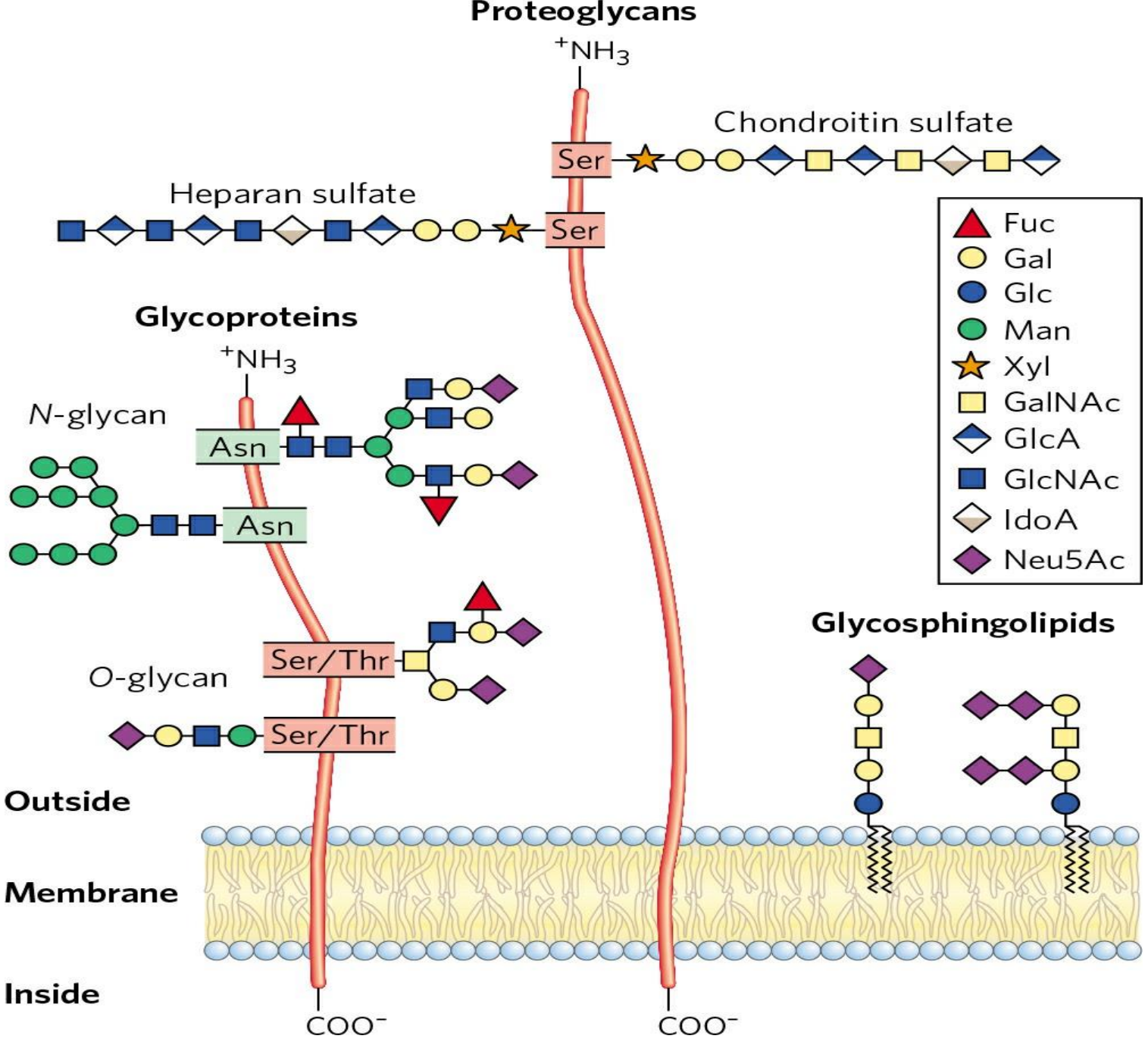
Proteoglycans are macromolecules of the cell surface or ECM in which one or more sulfated glycosaminoglycan chains are joined covalently to a membrane protein or a secreted protein. The glycosaminoglycan chain can bind to extracellular proteins through electrostatic interactions between the protein and the negatively charged sugar moieties on the proteoglycan.

Proteoglycans are major components of all extracellular matrices.

- Some provide communication between cells and their extracellular surroundings
- proteins for transport to and localization in specific organelles,
- for destruction when the protein is malformed ;
- recognition sites for extracellular signal molecules (growth factors, for example)
- extracellular parasites (bacteria or viruses).

- These oligosaccharides are central players in cell-cell recognition and adhesion, cell migration during development, blood clotting, the immune response, wound healing, and other cellular processes.
- In most of these cases, the informational carbohydrate is covalently joined to a protein or a lipid to form a **glycoconjugate**, which is the biologically active molecule


Proteoglycans
+NH3
Chondroitin sulfate
Ser
Heparan sulfate
Ser
Glycoproteins
+NH3
N-glycan
Asn
Asn
Ser/Thr
O-glycan
Ser/Thr
Fuc
Gal
Glc
Man
Xyl
GalNAc
GlcA
GlcNAc
IdoA
Neu5Ac
Glycosphingolipids
Outside
Membrane
Inside
COO−
COO−

Proteoglycans Are Glycosaminoglycan-Containing Macromolecules of the Cell Surface and Extracellular Matrix

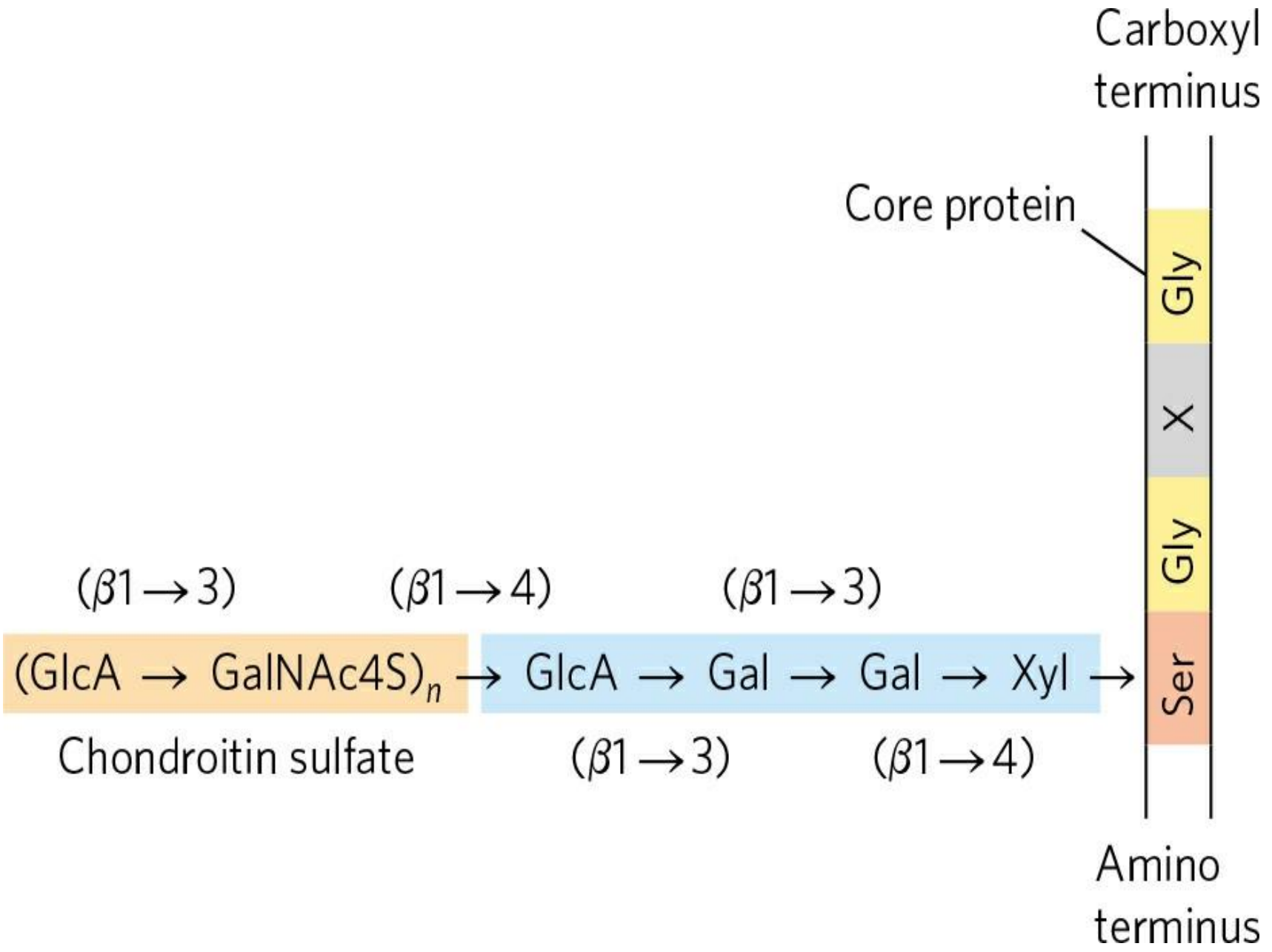
- Cells can produce at least 40 types of proteoglycans.

These molecules act as tissue organizers, and they influence various cellular activities, like

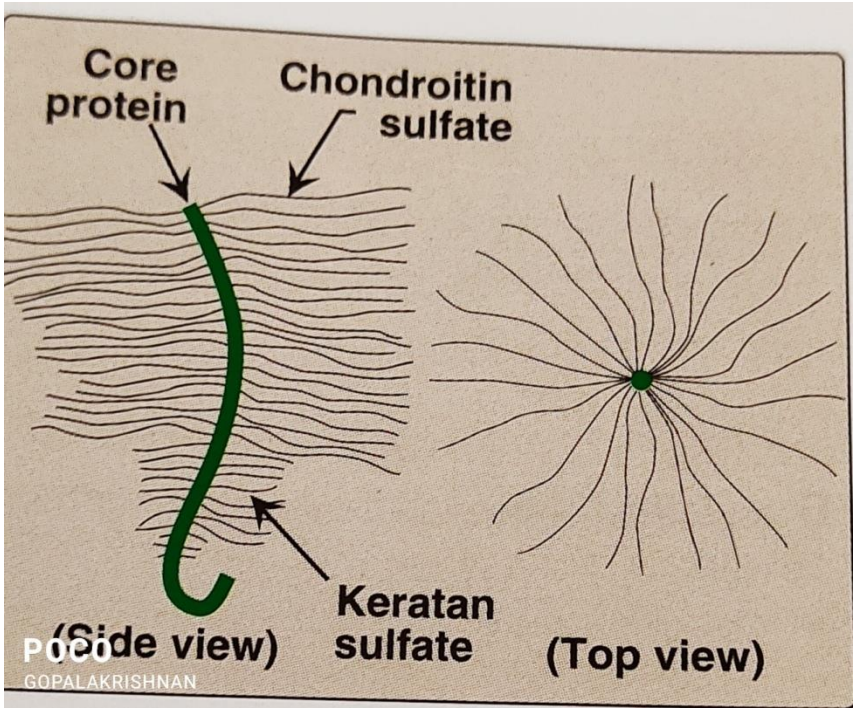
1. Growth factor activation and
2. Adhesion.

- The basic proteoglycan unit consists of a "core protein" with covalently attached glycosaminoglycan(s). The point of attachment is a Ser residue, to which the glycosaminoglycan is joined through a tetrasaccharide bridge . The Ser residue is generally in the sequence –

Ser–Gly–X–Gly– (where X is any amino acid residue) has an attached glycosaminoglycan

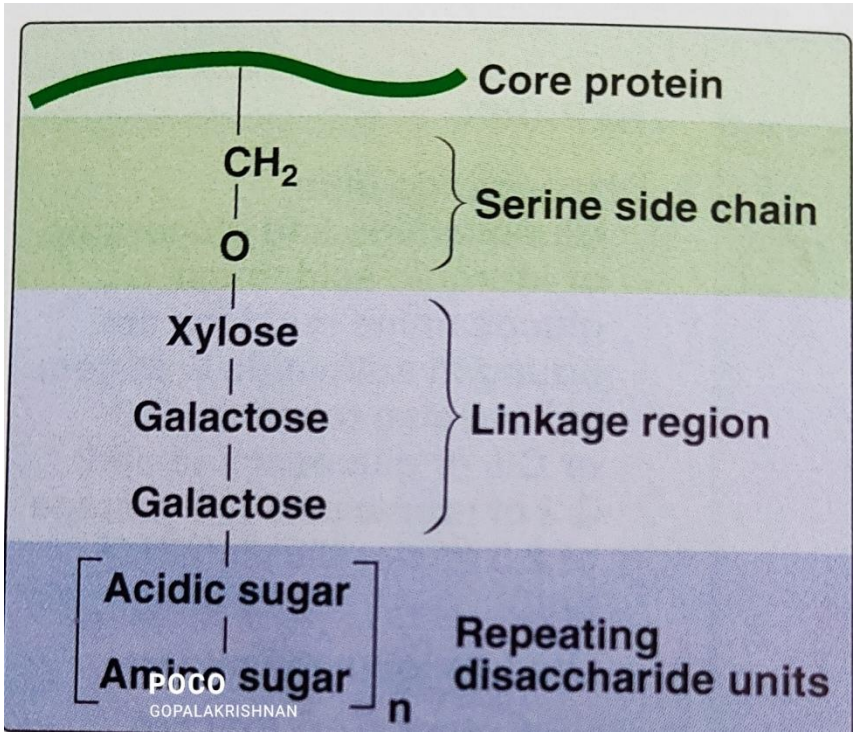

Carboxyl terminus
Core protein
Gly
X
Gly
Ser
(β1 → 3)
(β1 → 4)
(β1 → 3)
(GlcA → GalNAc4S)n → GlcA → Gal → Gal → Xyl →
Chondroitin sulfate
(β1 → 3)
(β1 → 4)
Amino terminus

- Monomer structure: A proteoglycan monomer found in cartilage consists of a core protein to which up to 100 linear chains of GAG are covalently attached. These chains, which may each be composed of up to 200 disaccharide units, extend out from the core protein and remain separated from each other because of charge repulsion. The resulting structure resembles a bottle brush

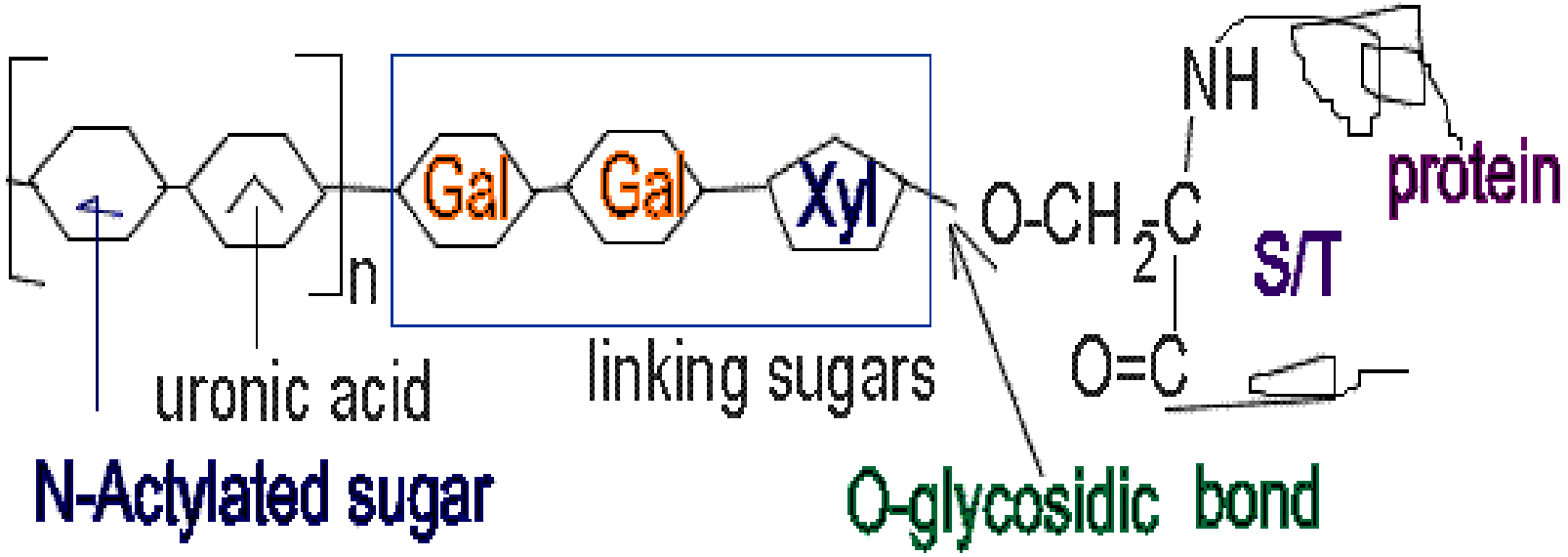


GAG-protein linkage

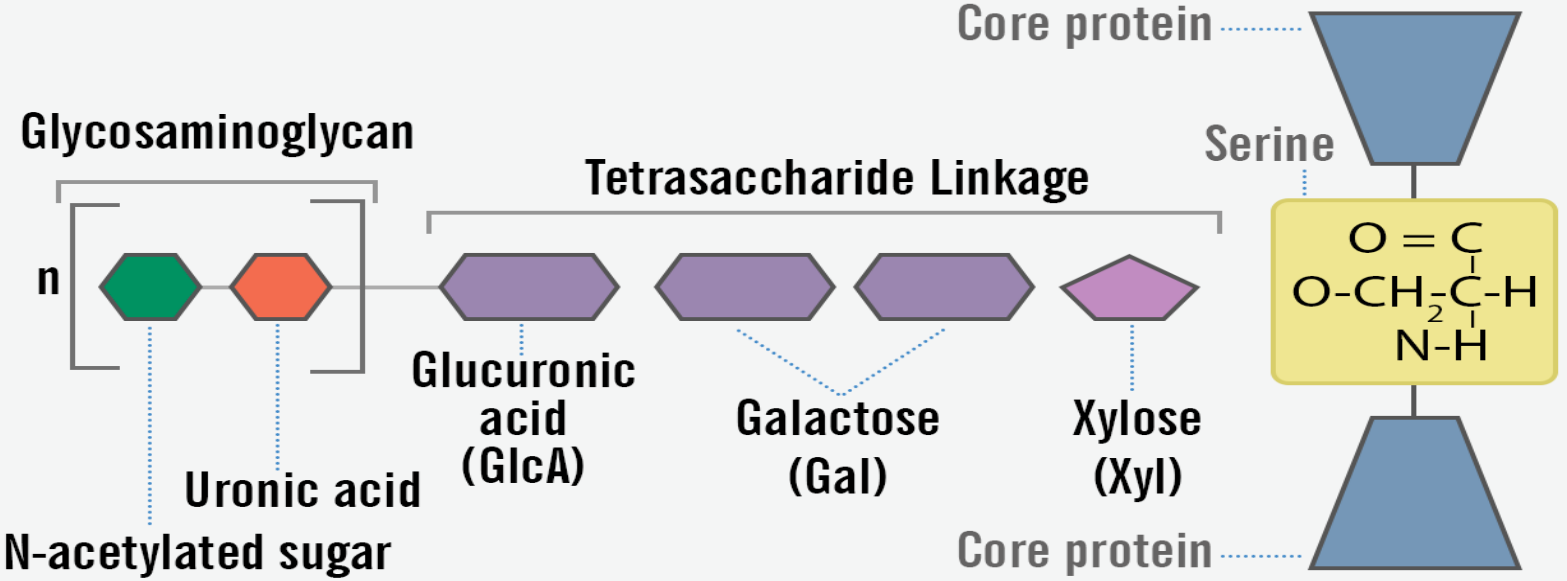
- This covalent linkage is most commonly through a trihexoside (galactose-galactose-xylose) and a serine residue in the protein. An O-glycosidic bond is formed between the xylose and the hydroxyl group of the serine

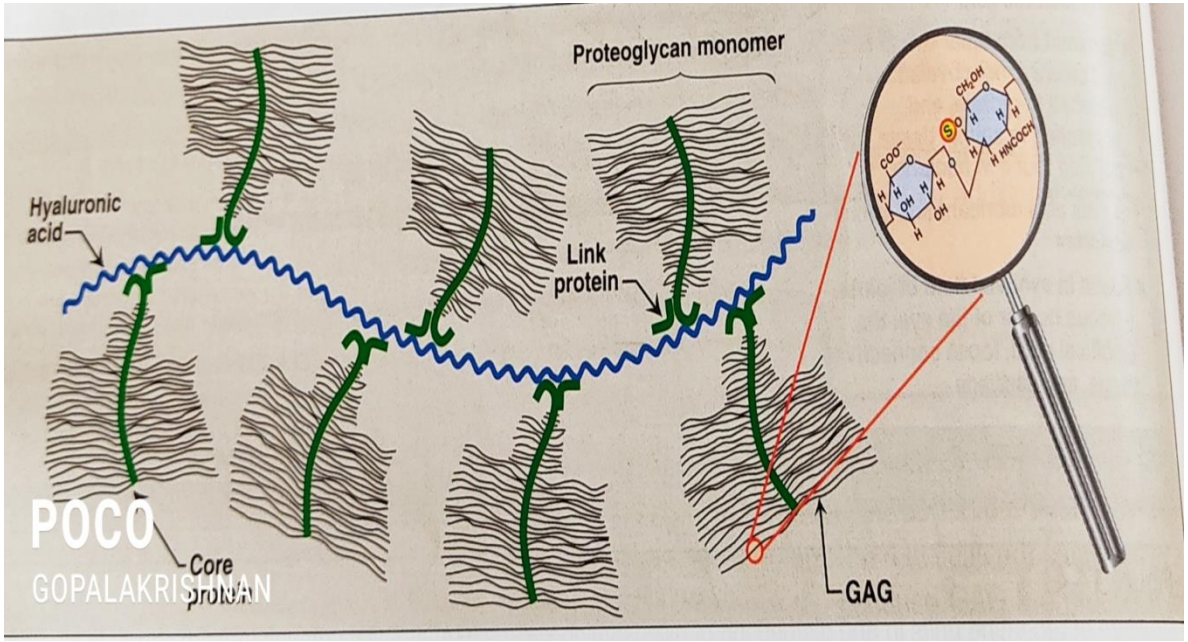


GAG LINKAGE TO PROTEIN IN PROTEOGLYCANS



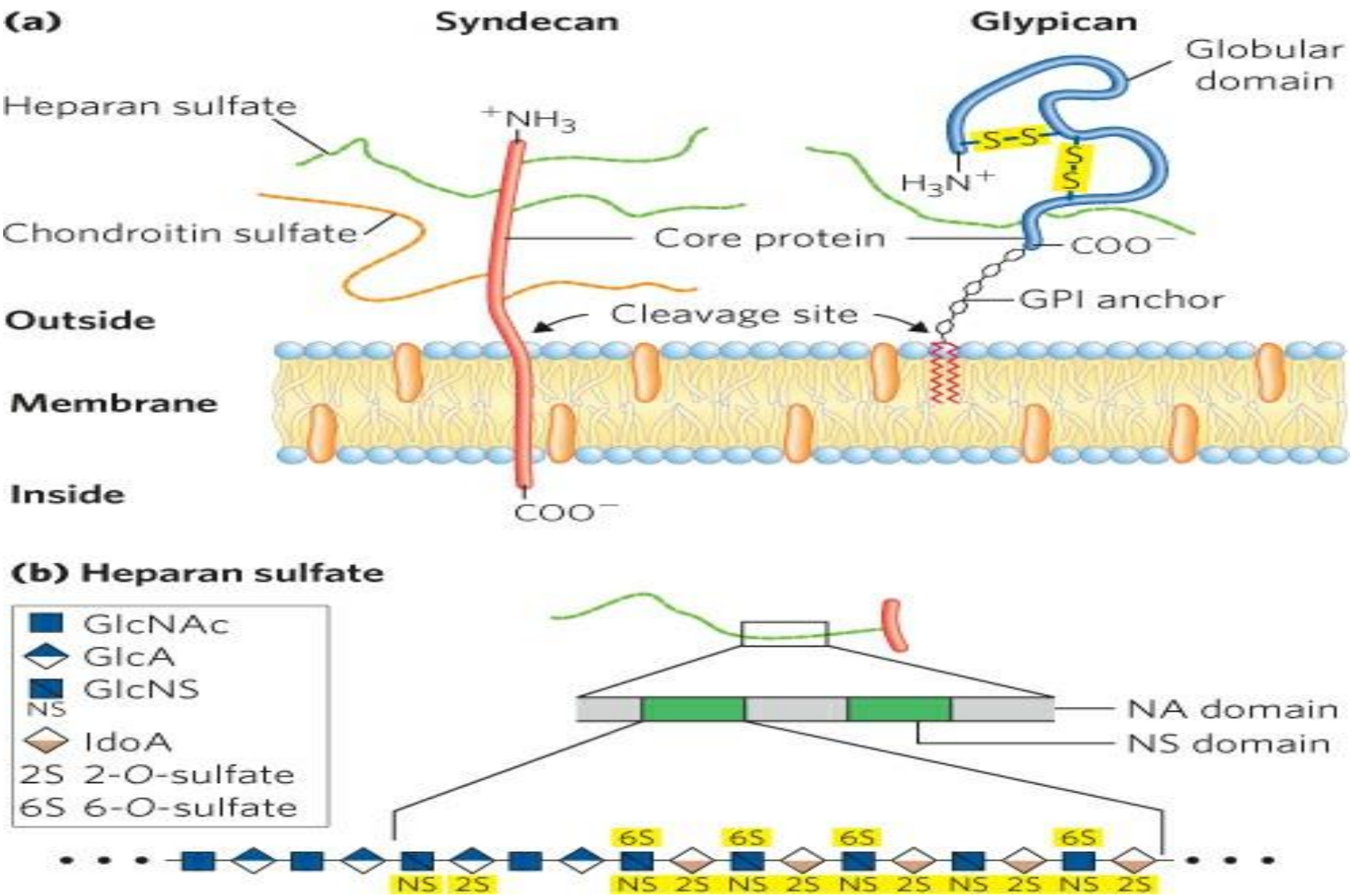
Basic Structure of Proteoglycans




Proteoglycan monomer
Hyaluronic acid
Link protein
Core protein
GAG
POCO
GOPALAKRISHNAN

- There are two major families of membrane heparan sulfate proteoglycans.
- **Syndecans** have a single transmembrane domain and an extracellular domain bearing three to five chains of heparan sulfate and, in some cases, chondroitin sulfate.
- **Glypicans** are attached to the membrane by a lipid anchor, a derivative of the membrane lipid phosphatidylinositol

- The glycosaminoglycan chains can bind to a variety of extracellular ligands and thereby modulate the ligands' interaction with specific receptors of the cell surface.
- Highly sulfated domains (called NS domains) , alternate with domains having unmodified GlcNAc and GlcA residues (*N*-acetylated, or NA, domains) .
- The exact pattern of sulfation in the NS domain depends on the particular proteoglycan; given the number of possible modifications of the GlcNAc–IdoA (iduronic acid) dimer

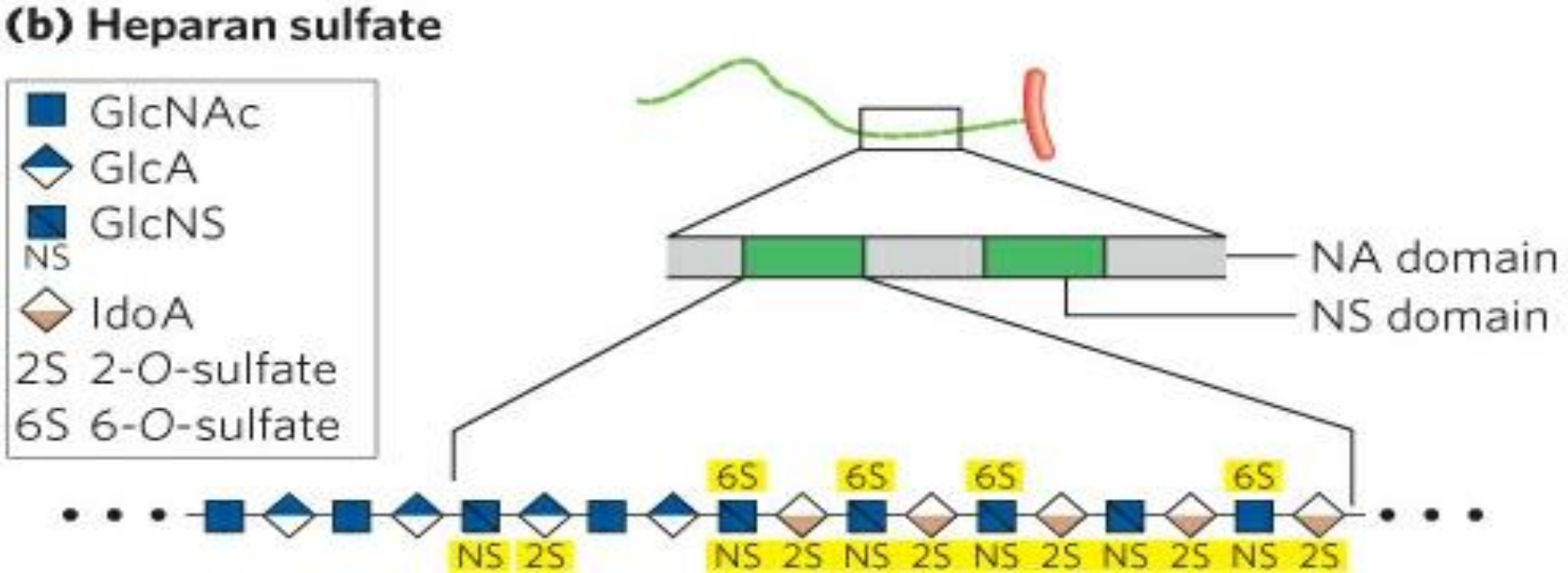

(a)
Syndecan
Glypican
Heparan sulfate
Chondroitin sulfate
+NH3
Core protein
Globular domain
S-S
H3N+
COO−
GPI anchor
Cleavage site
Outside
Membrane
Inside
COO−
(b) Heparan sulfate
GlcNAc
GlcA
GlcNS
NS
IdoA
2S 2-O-sulfate
6S 6-O-sulfate
NA domain
NS domain
6S
NS 2S

Two families of membrane proteoglycans

(a) Schematic diagrams of a syndecan and a glypican in the plasma membrane.

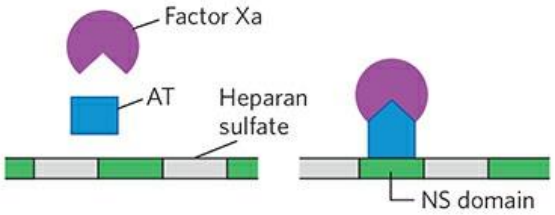
- Syndecans are held in the membrane through the hydrophobic effect by interactions between a sequence of nonpolar amino acid residues and plasma membrane lipids; they can be released by a single proteolytic cut near the membrane surface.
- In a typical syndecan, the extracellular amino-terminal domain is covalently attached (by tetrasaccharide linkers) to three heparan sulfate chains and two chondroitin sulfate chains.
- Glypicans are held in the membrane by a covalently attached membrane lipid (GPI anchor), but are shed if the bond between the lipid portion of the GPI anchor (phosphatidylinositol) and the oligosaccharide linked to the protein is cleaved by a phospholipase.

- **b)** Along a heparan sulfate chain, regions rich in sulfated sugars, the NS domains (green), alternate with regions with chiefly unmodified residues of GlcNAc and GlcA, the NA domains (gray).
- One of the NS domains is shown in more detail, revealing a high density of modified residues: GlcNS(*N*-sulfoglucosamine), with a sulfate ester at C- 6; and both GlcA and IdoA, with a sulfate ester at C-2. The exact pattern of sulfation in the NS domain differs among proteoglycans.



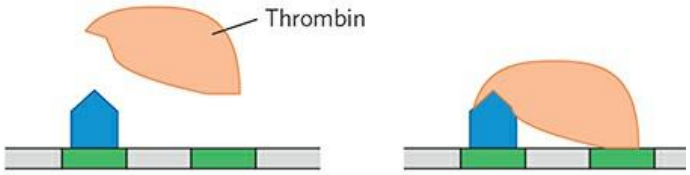
Four types of protein interactions with NS domains of heparan sulfate

(a) Conformational activation



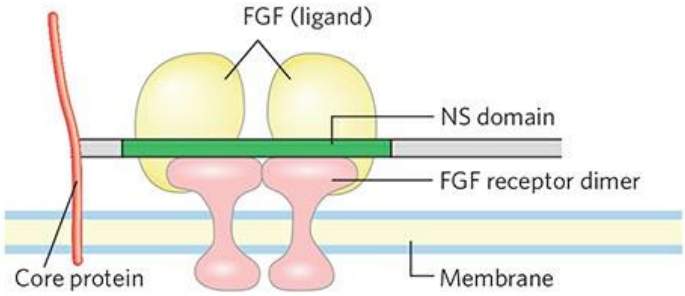
A conformational change induced in the protein antithrombin (AT) on binding a specific pentasaccharide NS domain allows its interaction with blood clotting factor Xa, preventing clotting.

(b) Enhanced protein-protein interaction



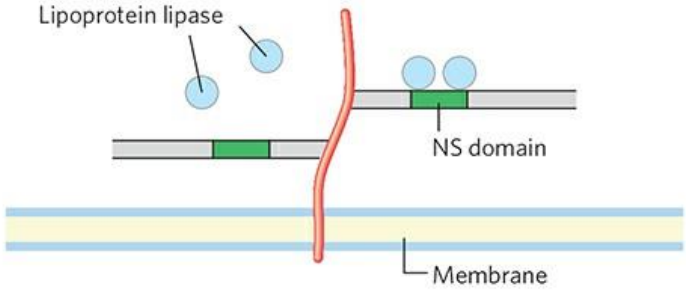
Binding of AT and thrombin to two adjacent NS domains brings the two proteins into close proximity, favoring their interaction, which inhibits blood clotting.

(c) Coreceptor for extracellular ligands



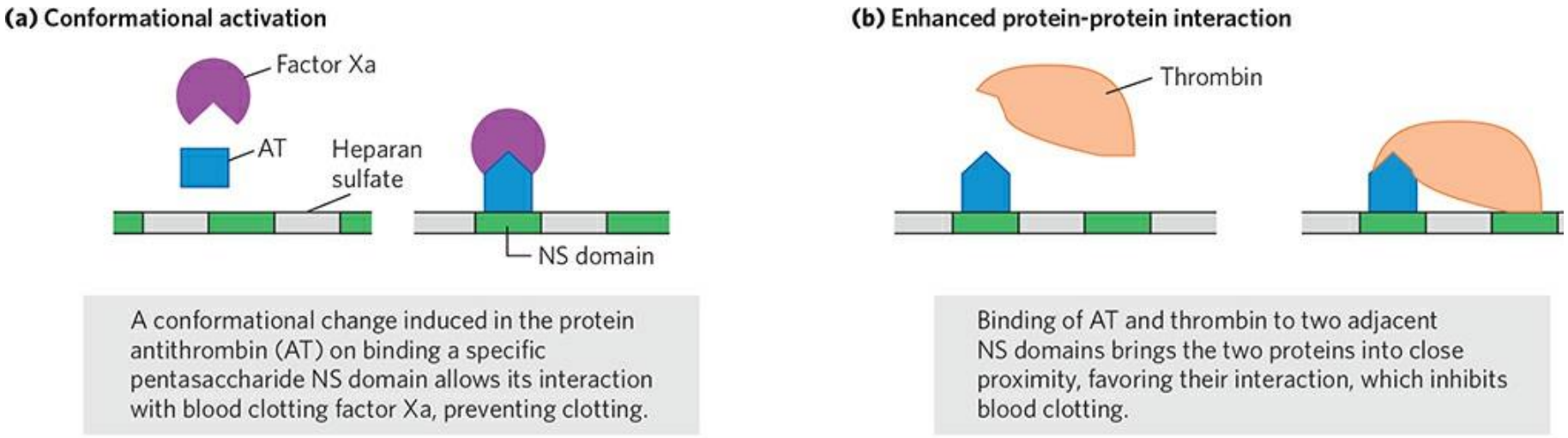
NS domains interact with both the fibroblast growth factor (FGF) and its receptor, bringing the oligomeric complex together and increasing the effectiveness of a low concentration of FGF.

(d) Cell surface localization/concentration

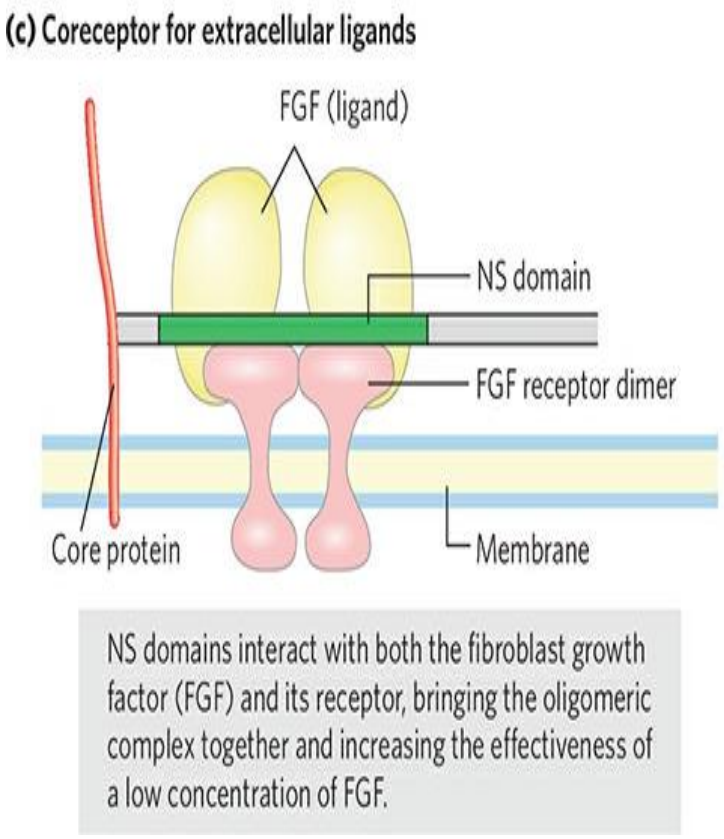


The high density of negative charges in heparan sulfate attracts positively charged lipoprotein lipase molecules and holds them by electrostatic and sequence-specific interactions with NS domains.

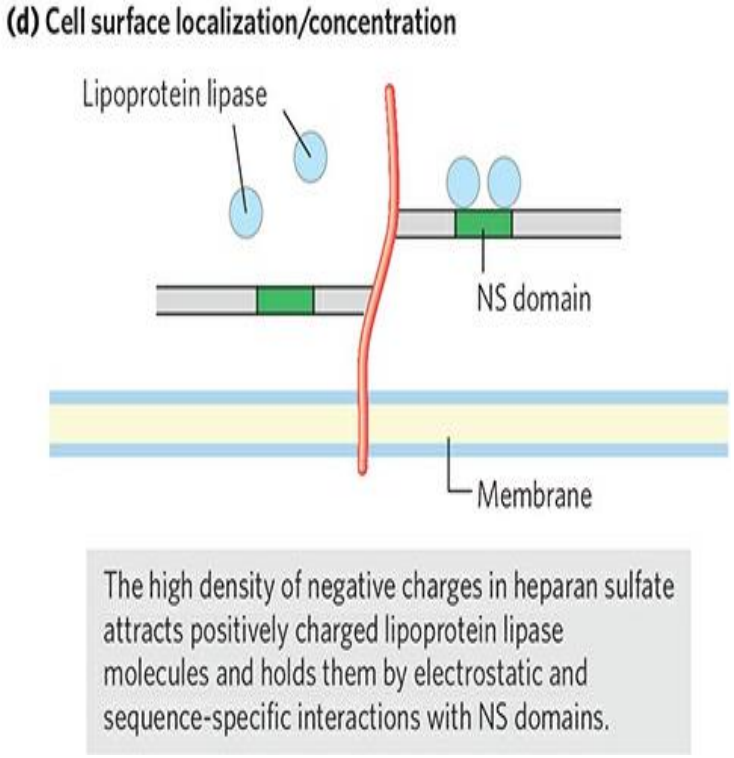
- A) Heparan sulfate molecules with precisely organized NS domains bind specifically to extracellular proteins and signaling molecules to alter their activities. The change in activity may result from a conformational change in the protein that is induced by the binding,
- B)It may be due to the ability of adjacent domains of heparan sulfate to bind to two different proteins, bringing them into close proximity and enhancing protein-protein interactions



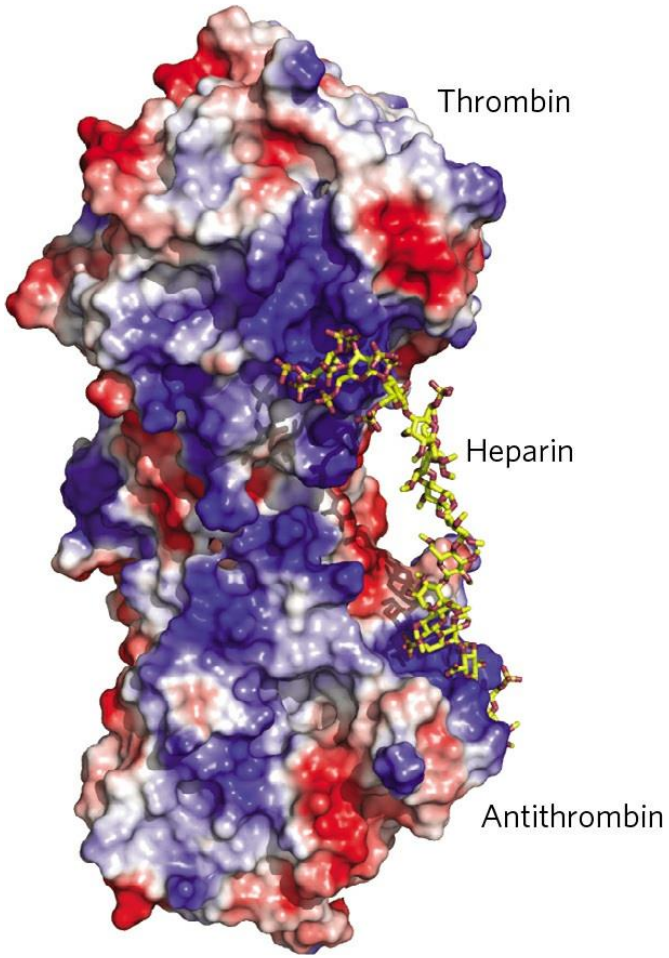
- C) The binding of extracellular signal molecules (growth factors) to heparan sulfate, which increases their local concentrations and enhances their interaction with growth factor receptors on the cell surface, the heparan sulfate acts as a coreceptor

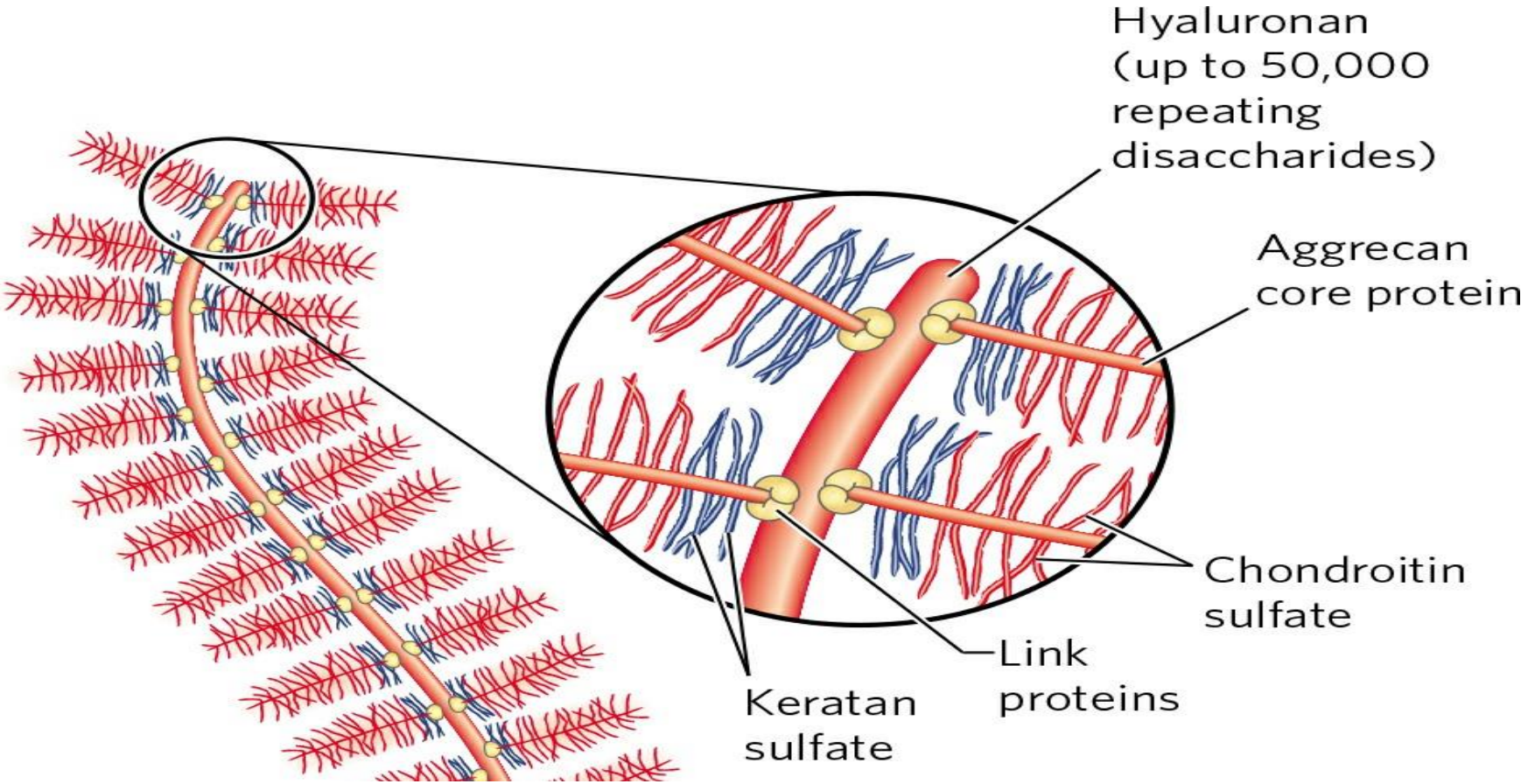


- Finally, the NS domains interact—electrostatically and otherwise—with a variety of soluble molecules outside the cell, maintaining high local concentrations at the cell surface.

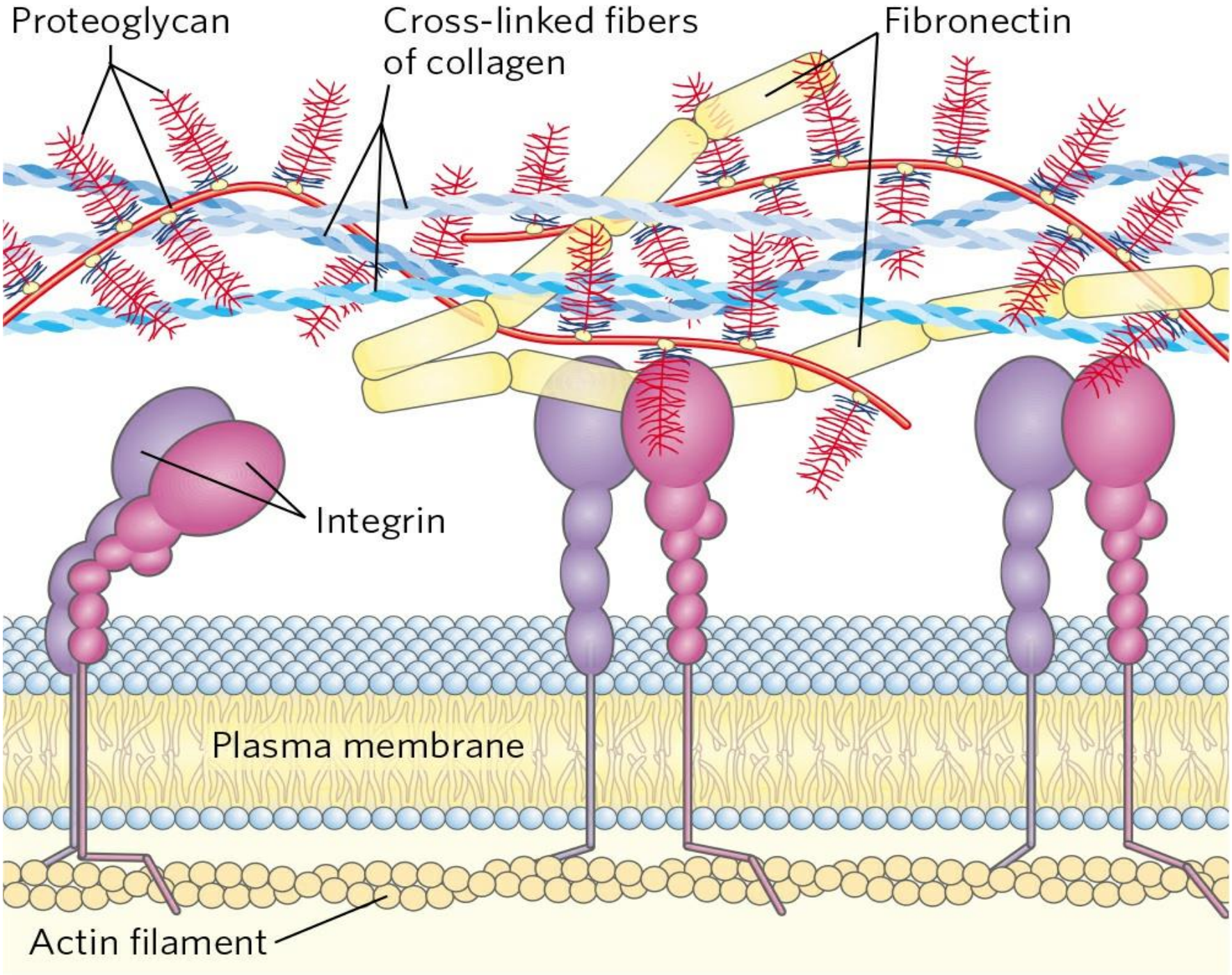


- The protease thrombin (blood coagulation) , is inhibited by another blood protein, antithrombin (prevents premature blood clotting).
- Antithrombin does not bind to or inhibit thrombin in the absence of heparan sulfate. In the presence of heparan sulfate or heparin, the binding affinity of thrombin for antithrombin increases 2,000-fold, and thrombin is strongly inhibited.
- When thrombin and antithrombin are crystallized in the presence of a short (16 residue) segment of heparan sulfate, the negatively charged heparan sulfate mimic is seen to bridge positively charged regions of the two proteins, causing an allosteric change that inhibits thrombin's protease activity .
- The binding sites for heparan sulfate and heparin in both proteins are rich in Arg and Lys residues; the amino acids' positive charges interact electrostatically with the sulfates of the glycosaminoglycans.




Hyaluronan
(up to 50,000
repeating
disaccharides)
Aggrecan
core protein
Chondroitin
sulfate
Link
proteins
Keratan
sulfate

- Some proteoglycans can form **proteoglycan aggregates**, enormous supramolecular assemblies of many core proteins all bound to a single molecule of hyaluronan.
- Aggrecan core protein (*M*r ~250,000) has multiple chains of chondroitin sulfate and keratan sulfate, joined to Ser residues in the core protein through trisaccharide linkers, to give an aggrecan monomer of *M*r ~2 × 106.
- When a hundred or more of these “decorated” core proteins bind a single, extended molecule of hyaluronate , the resulting proteoglycan aggregate (*M*r >2 × 108) and its associated water of hydration occupy a volume about equal to that of a bacterial cell! Aggrecan interacts strongly with collagen in the ECM of cartilage, contributing to the development, tensile strength, and resilience of this connective tissue.


Proteoglycan
Cross-linked fibers of collagen
Fibronectin
Integrin
Plasma membrane
Actin filament